

# MSGM Media Preparation

## MSGM Protocol:

1. Fill a beaker with MQ water to 90% of final volume
2. Add the chemicals in the specified order.
3. Do not mix all chemicals in powder form to then add the water. This seems to cause a problem for cell growth which we haven't figured out yet.

Chemical	per 1L (g)	per 4L (g)	Supplier
L-Ascorbic acid	0.035	0.14	Sigma, Cat.# A92902
Sodium acetate	0.07	0.28	Sigma, Cat.# S7545
Sodium thiosulfate	0.1	0.4	Sigma, Cat.# S7026
Sodium nitrate	0.12	0.48	Sigma, Cat.# 221341
Succinic acid	0.37	1.48	Sigma, Cat.# 398055
L-Tartaric acid	0.37	1.48	Sigma, Cat.# T109
Potassium phosphate monobasic	0.68	2.72	Sigma, Cat.# P9791
Wolfe's Mineral solution (-Fe)	5 ml	20 ml	"Homemade"

The recipe above is to prepare the basic solution of the MG medium. Vitamins and an iron source are missing from this and are added after autoclaving and just before starting cultures. For agar plates: let the medium cool down to ~45°C (hand warm) before adding additional components.

The following components are at a 100X concentration:

- 1) Wolfe's vitamins solution
- 2) Ferric malate

## Wolfe's Mineral Solution

Add nitrilotriacetic acid to approximately 500 mL of water and adjust pH to 6.5 with KOH to dissolve. Bring volume to 1L with remaining MQ water and add the following compounds one at a time (all reagents are from Sigma-Aldrich). To avoid errors make 4 L instead of 1 L.

Chemical	Cat. #	Amount per 1L
Nitrilotriacetic acid	N0128	1.5 g
MgSO <sub>4</sub>	M7506	1.465 g
MnSO <sub>4</sub> · H <sub>2</sub> O	M7634	0.5 g
NaCl	S6191	1.0 g

$\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$	255599	0.1 g
$\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$	C5080	0.1 g
$\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$	Z4750	0.1 g
$\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$	C7631	0.01 g
$\text{AlK}(\text{SO}_4)_2 \cdot 12 \text{H}_2\text{O}$	A7210	0.01 g
$\text{H}_3\text{BO}_3$	B0394	0.01 g
$\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$	M1003	0.01 g

Solution also available from ATCC (Cat. # MD-TMS; ~\$65 for 50 ml)

No need to sterilize. Wrap bottle with aluminium foil to protect from light exposure and store at room temperature. Good for a long time

### Wolfe's Vitamin Solution

3 mM Fe

9 mM Malate

Chemical	per 1L (g)	Supplier
Iron(III)chloride	0.486	Sigma Cat # F7134
DL-Malic acid	1.207	Sigma Cat # M0875

Make with MQ water. Filter sterilize solution by filtration through a Nalgene filter with 0.22 um pore size.

Wrap the container in which the solution is kept with aluminium foil to prevent light exposure and store at RT. Solution should be stable for long time.

Chemical	Supplier / Cat. #	Amount per 1L
Biotin	Sigma, B4501	2 mg
Folic acid	Sigma, 7876	2 mg
Pyridoxine hydrochloride	Fluka, 95180	10 mg
Vitamin B1 HCl	Fluka, 95160	5 mg
(-)-Riboflavin	Sigma, R9504	5 mg
Nicotinic acid	Fluka, 72309	5 mg

Calcium pantothenate	Sigma, C8731	5 mg
Vitamin B12	Sigma, V2876	0.1 mg
4-Aminobenzoic acid	Sigma, A9878	5 mg
(±)- $\alpha$ -Lipoic acid	Sigma, T5625	5 mg

Solution is also available through ATCC (Cat. # MD-VS; ~\$65 for 50 ml).